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CANCER AND VIRUSES*

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None of us has as many opportunities to speak to the public as we would wish. Since many of our conversations are with persons in the profession, we tend to forget that the language the profession uses is often a shortcut in communication and is not always easy to understand. I propose in this paper to make a serious effort to use plain English.

It was suggested that I deal with the subject of cancer and viruses. Before doing so, I wish to emphasize that the subject is not intended to imply that in human beings there is any known relationship between these two different things. I propose to demonstrate and hope to convince you that there is a relationship between cancer and viruses in animal species other than man. But I wish to state at the outset that direct evidence for an association between cancer and viruses in man is presently nonexistent.

It may surprise many to hear that it is more than 50 years since Peyton Rous, at The Rockefeller Institute here in New York, N. Y., discovered the first cancer virus of animals. So what we are to talk

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about is not something new. Some of it has been known for more than half a century. Rous made his classical discovery in 1911, but it was not then widely recognized as having broad significance. For a time it lay almost dormant and did not stimulate much work by others for nearly 20 years.

During the last 30 years, a number of other cancer-inducing viruses have been recovered from animals. This advance was initiated largely by the work of Richard Shope, also at The Rockefeller Institute, who first showed that several tumors of rabbits, including docile or benign tumors that do not kill, as well as some that do and are therefore called malignant, were caused by viruses.

At the present time we know of about 30 cancer-inducing viruses of animals. These viruses can lead to cancer in a considerable number of species, such as chickens that were first shown to have such viruses; frogs that may have cancer of the kidney; and numerous mammalian species such as mice, rabbits, rats, guinea pigs, hamsters, dogs, and cattle, including even monkeys. But as I said at the beginning, no virus is yet known to be directly related to cancer in human beings.

Under the circumstances, why is there so much interest—I think you will agree there is a great deal of interest as evidenced by many press reports—in the possibility that cancer in human beings may be associated with viruses?

There are four reasons for this interest. First, the frequency of association between viruses and cancer in a number of different animal species, either wild or domestic, has raised the possibility of a similar association in man.

Second, the types, sites, and sorts of cancer that animals have are similar to those of human beings in the majority of instances. As seen under the microscope, whether they have appeared in the mouse, rabbit, cow, or dog, it takes great skill to distinguish them from those of man.

Third, there is the possibility that early diagnosis, treatment, and prevention of cancer could be improved, perhaps strikingly, if viruses are, in fact, related to cancer in man.

Finally, vaccines effective against virus-induced leukemia of mice have been developed and are sufficiently effective in mice to be encouraging. This provides good reason to hope that some forms of human cancer may ultimately be found to be initiated by viruses.

Before going further, I think it would be of value to characterize the two words that represent the title of this lecture: cancer on the one hand and viruses on the other.

Let us begin with cancer, since this is what we are interested in most. Current concepts about cancer are not old. Many of the modern concepts are not more than about eight years of age. It is now widely recognized by most serious students of the disease that cancer is, in fact, a disease of individual cells. It represents a loss of the normal control of cells, particularly of cell growth. Such cells tend to run wild, without regard for other cells, and can destroy normal cells as well as their own kind if they be in the way. This concept has become sufficiently well established to make it possible to state that, if there were no cancer cells, there would be no cancers. The reason for emphasizing this is that it seems to me to indicate clearly where our studies should be directed. The cancer cell, in contrast to the recognizable tumor, is at the very heart of the problem. Clearly, if cancer cells could be eliminated, or if their development could be prevented, our problem would be solved.

Suppose now we ask what cancer cells are like, and how they differ from normal cells. During the last few years, largely as the result of the work of John Enders at Harvard University, Cambridge, Mass., it has become possible to cultivate almost any type of cell, whether animal or human, in the laboratory and to keep such cells growing more or less indefinitely.

As shown in Figure 1, I think you will perceive two clearly recognizable and different kinds of cells. Notice that some cells are in an orderly, mosaiclike array. They appear to have respect for each other. There are clear boundaries between the cell walls in each instance. In contrast to these cells there are others that are slightly smaller, stain a little more, and tend not to have much regard for each other. They may even pile up and grow on top of each other. These are the two kinds of cells that concern us. The first are normal human cells and the second are cancerous human cells, growing on a glass plate together. If we maintain this culture for some days, the cancer cells will migrate in all directions and crowd out the normal cells that are around them. The cancer cells even tend to disregard each other and show signs of aggression.

You will notice that each of the cells has two main parts. The cen-

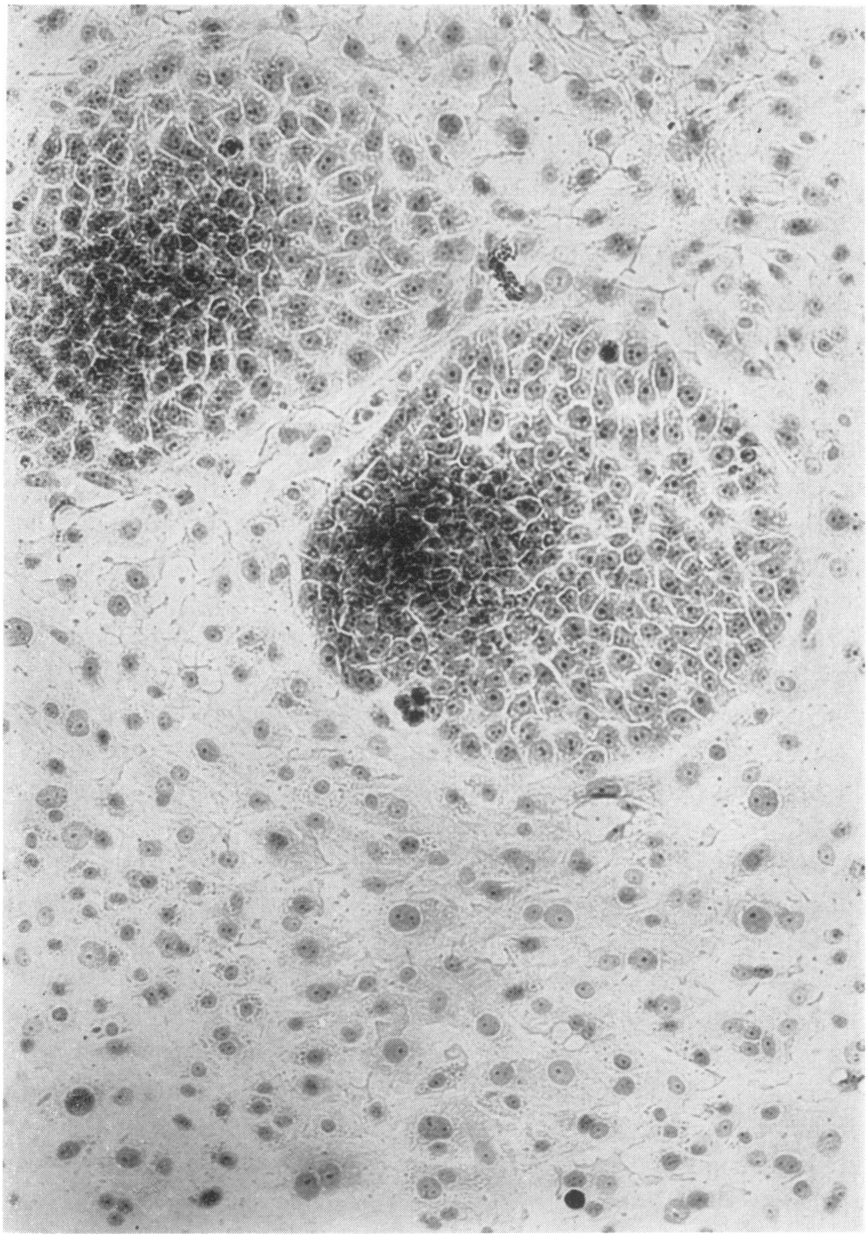

Fig. 1. Normal human cells and human cancer cells in culture. Two colonies of cancer cells are shown. The photograph was kindly provided by Dr. Jørgen E. Fogh.

tral or dark staining part is called the "nucleus." The outer part that does not stain so well is called the "cell body" or the "cytoplasm." The nucleus is the most important part, for it is in command, like the captain of a tiny ship. It guides and directs almost everything the cell does. The cytoplasm is comparable to a factory. It produces the new materials that the cell needs, chiefly proteins and enzymes, and controls the furnace of the cell, which consumes the food provided and releases the energy necessary for living things.

It is difficult to appreciate the dimensions of cells. They are very tiny and are seen only with a microscope. Each human being is composed of about 70,000 billion cells. This large number is the digit 7 followed by 13 zeros. Cells, whether normal or cancerous, multiply in the test tube at a fairly definite rate. They multiply by division and, after a few days in culture, each cell becomes two cells. Each new cell is identical to its partner, and both are identical to the cell from which they came. Normal cells in culture tend to produce normal cells, and cancer cells produce cancer cells and, in fact, can do so indefinitely. Human cancer cells have been grown in culture for about fifteen years and are still growing in many laboratories throughout the world.

The reason for emphasizing the long time that cancer cells can be cultivated is to make it clear that the cancerous change in cells persists. This change is transmitted continuously from one cell to another. This, in itself, would make one suspect that the change developed in what we may call "the captain of the ship" or the nucleus—the little central body—of the cell. As some of you are aware, there are 46 chromosomes in the nucleus of human cells, and we suspect that the change that characterizes cancer cells involves one or more of these chromosomes. This suggests that the cancerous change may involve the genetic apparatus of the cell, and there are good reasons to think that this may be so.

Normal cells can be made to become cancerous while in culture so that the process of change, or transformation, from normal to cancerous can be watched and seen to occur in a matter of a few days. This is a startling phenomenon to observe.

The simplest means by which to produce transformation is to use cancer-inducing viruses. For example, the virus discovered by Peyton Rous readily produces the cancerous change with appropriate cells in the test tube. So, too, with several mouse viruses and even with a monkey virus. But perhaps most surprising is the finding that normal human

TABLE I—CANCER DEATH RATES, 1960-62, AGE-ADJUSTED

	Male			*Female*	
Order	*Site*	*Rate**	*Order*	*Site*	*Rate**
1	Lung	33.4	1	Breast	22.2
2	Colon-rectum	18.8	2	Colon-rectum	17.0
3	Prostate	13.2	3	Uterus	13.2
4	Stomach	11.8	4	Ovary	7.6
5	Pancreas	8.2	5	Stomach	5.9
6	Leukemia	7.5	6	Leukemia	4.8
7	Esophagus	3.8	7	Lung	5.0
8	Kidney	3.2	8	Pancreas	4.8

*Rate per 100,000 population. Source: American Cancer Society, New York, N. Y.

cells can be transformed to the cancerous state in culture and remain cancerous after this artificial transformation has occurred. This was shown by John Enders and his associates at Harvard, who employed the monkey virus—SV40, as it is called. This must not be thought to suggest that this virus would lead to cancer in an intact human being. We are quite certain that it does not. However, in the test tube, with cells separated from the body and free from all normal controls, the transformation does, in fact, occur.

Cells that are made cancerous in the laboratory—cells that are changed on purpose—have the same features, so far as we can determine, as naturally occurring cancer cells. Indeed they pass the ultimate test—the only test that is reliable when one suspects the cancerous change has occurred—and lead to cancer in animals when they are inoculated.

One frequently hears it said that cancer is not one disease but many—perhaps a great many—different diseases. I shall take the attitude this evening that cancer is not many diseases but instead is only a single disease. The different types, of which there may be several hundred, are simply evidences of the same cellular change initiated in different kinds of cells, in different sites, and in different organs. But the underlying cell change seems to be similar in all instances.

Table I lists some of the different types of human cancer, and the frequencies of occurrence at various sites in men and women. The figures shown are cancer death rates. We cannot give morbidity or illness rates, because they are simply not available, and so we must use these distressing figures, which are age-adjusted* for the period 1960 to 1962. My reason for presenting these data is to show how dissimilar the sites are in human beings in relation to sex. You will see that cancer of the lung is the most common type for men but stands in seventh place for women; that cancer of the breast is the most common type for women and is not even on this list, although it does occur rarely in men. Only cancer of the bowel—colon and rectum—has approximately the same frequency in both sexes. Nearly all the other major sites listed have different frequencies in men and women.

The different frequencies of cancer sites in men and women reflect the importance of genetic constitution in this disease, since sex, as you know, is genetically determined and attributable to but a single pair of the 46 chromosomes in human cells.

Of most concern is the nature of the cancerous change in cells that leads previously normal cells to this enduring malignant state. The change seems to correspond to what can be described as a mutation of cells—a persisting variation—as an example, the kind of thing that has led to the development of so many different breeds of dogs. Dogs are of one species. They interbreed, but clearly there are large differences between a Boston terrier and a Great Dane although both are, in fact, dogs. These differences have resulted from mutations that have been selected over the years and, to some extent, the differences between normal cells and cancer cells are comparable.

The new and malignant features of cancer cells are permanent and remain present indefinitely no matter how many generations of the cells are cultured in the laboratory. They appear to be regularly transferred to each of the daughter cells—I wish to emphasize the word cells—because I do not want to raise any suggestion that they are transmissible from parent to child. For such a possibility there is no reliable evidence.

We can turn now from the first word of the title to the second, that is, viruses. We should ask, I think, what they are and how they act. Viruses are the smallest of known infectious agents but have no life of their own and can multiply only inside living cells. The cells may be bacterial, or plant, or insect, or animal, or those of a human being. Several thousands of viruses are now known. Several hundreds can infect human beings. To give an idea how tiny they are by stating their dimensions in millionths of an inch would not have much meaning. I

*Adjustment of crude death rate to take account of changes in composition of population.

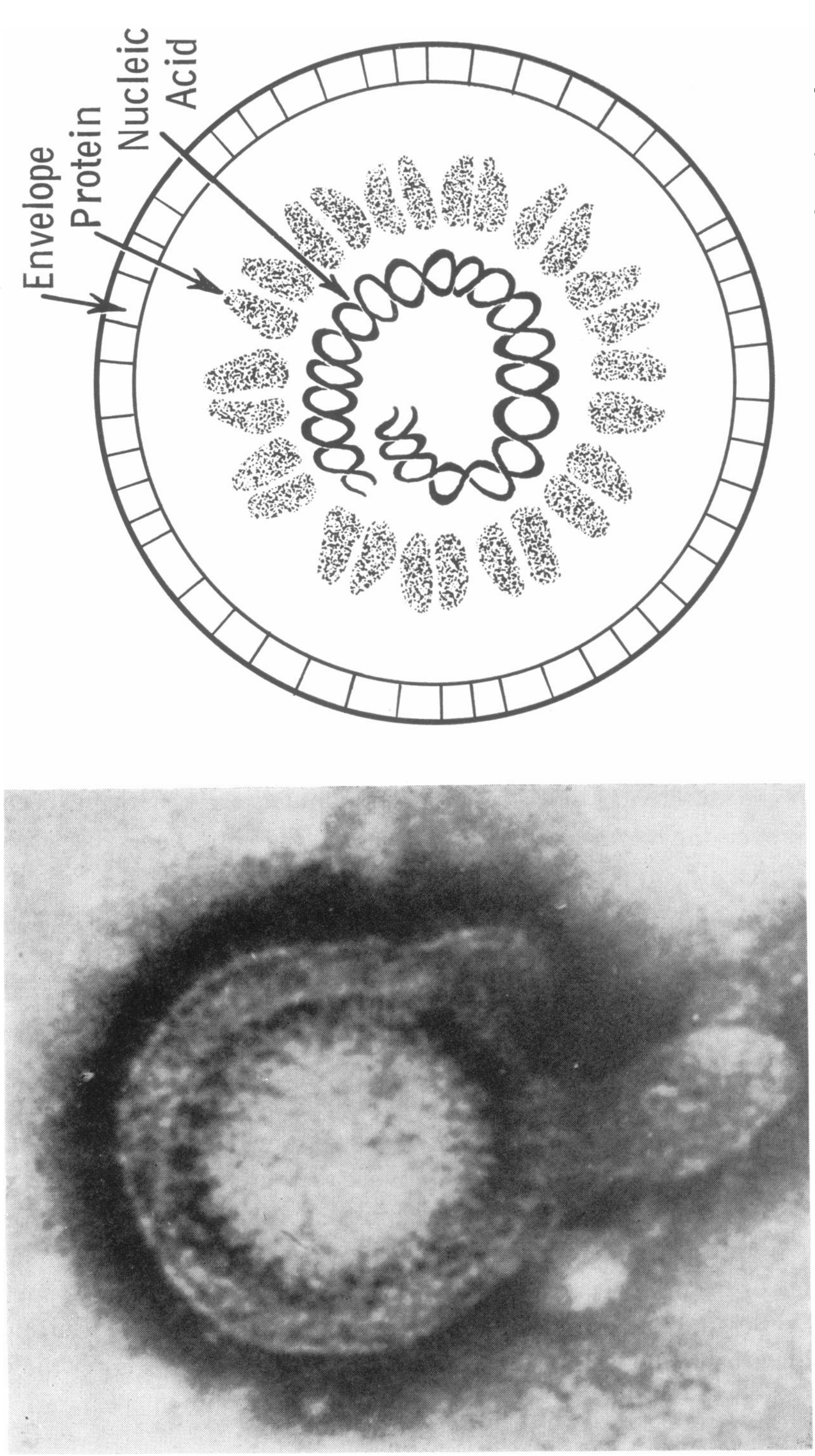

Fig. 2. A virus particle and a diagram of its major components. The virus is called herpes simplex. This photograph, made with an electron microscope, was published previously by P. Wildy and D. H. Watson, *Symposia on Quantitative Biology*, vol. 27, 1962, and is reproduced by permission.

said earlier that each human being is composed of approximately 70,000 billion cells. A single cell can support the growth and multiplication of as many as 20,000 poliovirus particles.

Figure 2 shows a photograph of a virus particle, about which I shall have more to say in a moment, and a diagram of the same virus particle. We see its form as visualized in the electron microscope and its composition in terms of the major components. This is a typical small spherical virus particle, and the schema on the right indicates of what the particle is composed. The original magnification of this virus particle in the electron microscope was 160,000 times larger than the particle. As shown on the large screen, the magnification is about 3-millionfold. Virus particles are so tiny as to be visualized only with the electron microscope.

As it happens, this is not a cancer-inducing virus. It is the virus that causes fever blisters and is called herpes simplex. But I doubt that anyone, including the best electron microscopists, could distinguish it from other viruses that appear very much like it, are composed of the same constituents, and do, in fact, induce cancer in animals.

You will see from the diagram that at the outside of the particle there is an envelope or membrane. Within this envelope are the major components. These are a protein layer that covers an inner core of nucleic acid. The protein layer is an irregular coatlike mass, and the inner core is a helix—a double corkscrew—of nucleic acid. The core, the central part, is composed of DNA, the nucleic acid that is referred to so often in the press. It is the genetic component of the virus, for viruses, tiny though they are, have their own special genetic apparatus. This nucleic acid core can guide and even control certain activities of a cell that is infected by the virus and can lead to the production of many more virus particles.

The protein layer, the irregular coat around the internal core, is different from the proteins of the cell and is recognized as being different by the infected animal or human being. Being different, it stimulates antibody production and other immune reactions.

Many different viruses are known, probably several thousand in total, and they may infect any species of living organisms from bacteria to the largest animals, including human beings. As many as 300 different types of viruses have been identified that can infect human beings. As stated earlier, about 30 viruses are known to be capable of inducing

TABLE II—SOME TUMOR-INDUCING VIRUSES

Year	*Tumor*	*Virus*	*Species*	*Investigator*
1911	Sarcoma	RSV	Chickens	Rous, P.
1919	Wart	Wart	Man	Wile, U. and Kingery, L.
1933	Wart	Papilloma	Rabbits	Shope, R.
1933	Lymphomatosis	Avian	Chickens	Furth, J.
1934	Cancer (skin)	Papilloma	Rabbits	Rous, P. and Beard, J.
1936	Cancer (breast)	Milk	Mice	Bittner, J.
1938	Cancer (kidney)	Frog	Frogs	Lucké, B.
1951	Leukemia	Leukemia	Mice	Gross, L.
1957	Many types	Polyoma	Mice	Stewart, S. *et al.*
1962	Sarcoma	SV40	Hamsters	Eddy, B. *et al.*
1962	Sarcoma	Adeno-12	Hamsters	Trentin, J. *et al.*
1963	Sarcoma	RSV	Monkeys	Munroe, J. and Windle, W.

cancer in animals of various species.

Among the viruses that are known, I have selected about a dozen that can induce tumors in animals, as shown in Table II. One of these, the wart virus, does not induce cancer and is included for other reasons. A wart is not a cancer, either in a man or a rabbit, but warts are included because they represent the only tumor of human beings that has been proved so far to be caused by a virus. This group of 12 viruses serves two purposes. First, they represent some of the most important milestones in this field on historical grounds. Second, they indicate the wide variety of cancer types that can be induced in various animal species by viruses.

A few brief comments on some of these viruses may reveal some important relations that are not immediately apparent. In the first place, with the sarcoma virus of chickens you will see that it may also induce cancer in monkeys. Monkeys, of course, are primates and are distantly related to man on evolutionary grounds. Not only will some strains of RSV induce cancer in chickens and monkeys, but also they may induce cancer in rats, hamsters, and guinea pigs. This negates a concept that was held for many years and clearly indicates that some viruses may be capable of inducing cancer in several different animal species. This will become even more evident with the polyoma–many-tumor–virus of mice.

I emphasized earlier that the only tumor of man that has been shown to be caused by a virus is the common wart. Rabbits also have warts or papillomas, and these were shown by Richard Shope to be caused by a virus. Ultimately it was found that some cancers in rabbits resulted from the same virus that induced warts. This does not suggest that warts in man may lead to cancer. There is no evidence that they do.

The next virus I wish to bring to your attention is that discovered by the late John J. Bittner at the Jackson Memorial Laboratory, Bar Harbor, Maine. He found that there was in the milk of some female mice an agent that we now know to be a virus and that this agent could lead to the development of breast cancer in the female offspring of such mice. No similar virus has been demonstrated in human milk or in the breast cancers of women, and there is no reliable evidence to support the idea that such an agent is related to this type of cancer in human beings.

The frog kidney cancer is mentioned only to show how wide is the spectrum of species that are susceptible to cancer-inducing viruses. I should point out that there is also a virus of certain plants—the "wound tumor virus"—that can cause tumors even in them.

Beginning little more than a decade ago this field rapidly expanded, and many of the cancer-inducing viruses have been discovered since 1951. Ludwik Gross, at the Veterans Administration Hospital, Bronx, N. Y., first showed that leukemia of mice was, in fact, due to a virus. In order to accomplish this, it was necessary to inoculate newborn mice soon after birth. Newborn animals are immature in many ways, particularly with respect to their immune responses and, instead of reacting against a foreign agent of this kind, may accept and continue to maintain it.

It seems probable now that most, if not all, leukemias of mice are caused by viruses. There are several different types that can be identified by relatively simple tests in the laboratory. These virus types may be thought of as comparable to the well-known types of other viruses: for example, the three types of poliovirus.

The virus that led to a change in many early theories about this problem is the "many-tumor" virus—polyoma—of mice, which was identified in 1957. This is the virus that settled the argument that, if there were viruses that caused cancers, there would be a separate virus for each type.

Polyoma virus can lead to some 20 different types of cancer in at least six different animal species and has induced several types of cancer in a single animal. This is part of the evidence that supports the hypothesis I stated earlier: that cancer is, in fact, but one disease entity that may be expressed in many different types and sites. Polyoma virus can initiate cancer in many different glands, the skin, the intestine, the breast, muscle, bone, and several other tissues. This one virus can induce cancer in mice, hamsters, guinea pigs, rats, and some other animals. Of even more interest, the little core in the center of the virus particle shown in Figure 2—the nucleic acid—is of the DNA type in polyoma virus. This nucleic acid core can be extracted from the virus and the DNA alone will still lead to cancer after injection in newborn animals.

This finding clearly points to the central role of the genetic apparatus of cells in relation to that important question: What is the nature of the cancerous change in cells? Recent evidence suggests that the core, the nucleic acid of the virus particle, may become associated with the chromosomes of the animal cell and that this association of the two different kinds of genetic material may lead to the cancerous change in the cell.

I mentioned earlier that human cells contain 46 chromosomes. Certainly you have seen articles in the newspapers about the remarkable properties of the nucleic acid, DNA, the genetic molecule. But few realize that the 46 chromosomes of a human cell may contain about 60,000 molecules of DNA. The dimensions of the problem now become clearer, for it is possible that only a few of the approximately 60,000 molecules of DNA need to be much altered to account for the cancerous change in cells. This may, in fact, prove to be similar to the problem of the needle in the haystack, and it may take much time and much effort to find what and how the DNA molecules are altered.

I draw your attention now to SV40 virus and adenovirus type 12, which were discovered to be cancer-inducing in animals in 1962. These two viruses have been particularly revealing and, for a time, were a little disturbing. SV40 virus is present in the kidneys of a small percentage of apparently normal monkeys. In monkeys it causes no recognizable disease and probably does not induce cancer. But if it is injected into newborn hamsters, it leads to cancer promptly and with high frequency. This indicates more than success in simply moving a virus from one species to another. This virus, which is not disease-producing in its natural host, when transmitted to an unnatural host initiates the development of cancer. But even more surprising is the virus identified as adeno-12. This is, in fact, a human virus—one that may cause only mild upper respiratory infection similar to the common cold in human beings. It does not lead to cancer in human beings and the evidence for this is extensive. Nonetheless, when it is transmitted to an unnatural host —the newborn hamster, rat, or mouse—it is effective in inducing cancer in these animal species.

With so much known now about cancer in animals, why has it not been possible to obtain equally decisive evidence with human cancer? I think some reasonable answers can be provided to such a question. Throughout this century, indeed beginning within two years of discovery of the first animal virus in 1898, there have been innumerable attempts by many of the best workers in the field to discover whether viruses were, in fact, associated with human cancer. There is no great difficulty about finding common and well-known viruses in human cancers. Many have been recovered and are undoubtedly simply passenger viruses going along for the ride, if you will. None has yet been decisively associated with cancer in human beings. When you recall that there are no fewer than 60 different viral types that can be isolated from the intestinal contents of human beings and that there are more than 80 different viral types that can be recovered from the respiratory tract—the nose, throat, and bronchi—of human beings, it is perhaps not surprising that similar viruses should also be found at times in human tumors.

The difficulty of identifying cancer-inducing viruses in animals is large, but one can do experiments with animals that could not conceivably be carried out with human beings. For example, I have emphasized that several of the viruses were found to initiate the development of cancer only when they were inoculated in newborn animals.

A major difficulty both in man and in animals is due to the unique relationship between viruses and cancer cells. In the classical infectious diseases—measles, influenza, poliomyelitis, the common cold—the viruses do not disappear but multiply and increase in numbers greatly during the course of the illness. But viruses frequently disappear as infectious agents when they change cells from normal to cancerous. You will recall that the isolated core—the nucleic acid—of polyoma virus, can itself lead to cancer. It is thought that the viral core may become associated with the genetic apparatus of the cell. Under these circumstances, it seems to be unable to produce more infective viral particles, but it may add abnormal genes to the genetic machinery of the cell in which it is lodged. The cells that become cancerous may not yield infective viral particles at all and, therefore, the virus cannot be found by usual techniques. To detect it even in animals has often required highly artificial conditions and, unless the investigator creates such conditions, no virus may be found.

Polyoma—many-tumor—virus infects many mouse colonies but, under natural conditions, appears not to induce cancer. Nonetheless, it has the potentiality of inducing as many as 20 different kinds of cancers when the investigator gets between the mouse from which it came, and the mouse to which it goes. In nature most potential cancer-inducing viruses do not lead to cancer but only to ordinary infectious diseases.

To demonstrate the many different cancer viruses known in animals, investigators have employed artificial conditions such as immature animals, which do not have normal immune responses, cultured cells of animal embryos where all the normal bodily influences, whether hormonal, immune, or nervous, are absent, and several others. In these various ways experimenters have been successful in recovering cancer-inducing viruses from animals.

There is a possibility that human cells cultivated in the laboratory may be susceptible to some of these hypothetical agents. This has been extensively tested but so far has not been successful. At the moment, it is vastly easier to prove that a particular virus recovered from human beings is not cancer-inducing than it is to establish decisively that it is causally associated with human cancer.

Electron-microscope studies that do not depend on any biological activity but simply visualize viruslike particles have been carried out by many experts on many types of cancer, including those of human beings, for more than 10 years. There is no doubt that particles of size and form similar to virus particles have at times been seen but, in my view, such evidence is not decisive. We still adhere to the view that the only completely reliable criterion for a cancer-inducing virus is the demonstration that it can induce cancer in the laboratory under carefully controlled conditions.

Various particles, some of which appear similar to those of certain viruses, have been found with the electron microscope in the blood of some patients with acute leukemia. Some optimistic statements have been made about what this could mean were they, in fact, virus particles. But there are many uncertainties between visualizing such particles and demonstrating that they are viruses, on the one hand, and will induce cancer on the other.

Finally, in brief conclusion, the cancers of animals do not seem to be different in any significant way from those of human beings. They are of the same types and may occur in the same sites. In some animal species they develop with frequencies similar to those seen in human beings. The cancers of animals are, frequently, attributable to viruses. Indeed, it appears that, in those instances in which the primary incitant of naturally occurring animal cancers has been demonstrated, it has turned out to be virus.

The number of cancer-inducing viruses and the number of species affected by them are sufficiently large to suggest that, unless one takes a rather forced point of view and insists that cancers of human beings are unique, it would be difficult to defend the idea that viruses do not have anything to do with some human cancers.